Nuclease

Week 8

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

This text will be like an abstract or summary of the whole week. This way to reader will understand what has been done this week and what results were conducted, before entering each experiment. OBS! Might not be needed as we will have a summary on the wiki page for each week.

Definitions

BL21(DE3)

Top10

PCR

ddH_2O

T4 DNA ligase

T7

pSB1C3-Nuc

XbaI

PstI

pSB1C3-T7-Nuc

pSB1C3

ATP

EcoRI

PstI

pSB1A3

1 Digestion of pSB1C3-Nuc PCR product (from week 5) with gel purification

Responsible

Ellinor Lindholm, Oscar He and Maren Maanja

Protocols used

3A Assembly (Sigma)
Gel purification

Modifications and comments to protocols

3A Assembly (Sigma): total volume 10 μl (digestion mix)
3A Assembly (Sigma): 1 μg of DNA in digestion mix

Experimental Setup

Table 1: Concentrations of the nuclease plasmid samples used for digestion.

Sample	Concentration [ng/μl]
Nuc 2.1	75.8
Nuc 2.2	73.8

Table 2: Volumes of digestion mix components for both samples.

Component	Nuc 2.1 [μl]	Nuc 2.2 [μl]
DNA sample	6.6	6.78
10x SuRE/Cut Buffer H	1	1
XbaI	0.5	0.5
PstI	0.5	0.5
ddH_2O	1.4	1.22

Table 3: Weights of the cut out gel bits and volumes of Buffer QG and Iso-propanol used for the gel purification.

Sample	Weights of gel cut out [g]	Buffer QG [μl]	Iso-propanol [μl]
Nuc 2.1	0.0588	176.4	58.8
Nuc 2.2	0.0533	159.9	53.3

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (1)$$

Results and Conclusions

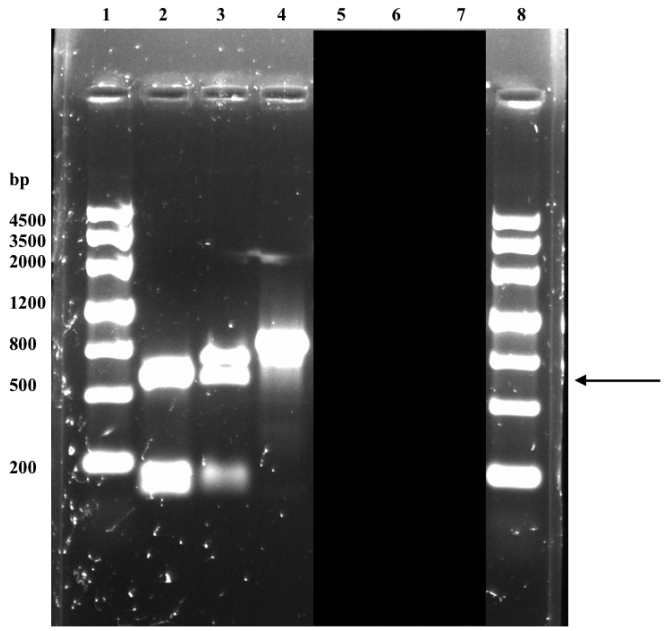

Figure 1: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 2.1 (3) Nuc 2.2 (4) Negative control (8) DNA Ladder.

The digestion of pSB1C3-Nuc was successful (expected size 561 bp).

Table 4: Concentrations of gel purified samples.

Sample	Concentration [ng/μl]
Nuc 2.1	2.2
Nuc 2.2	-0.8

The purification was successful. Sample Nuc 2.2 was discarded since the concentration turned out negative, possibly because no DNA was present as measured by NanoDrop.

Discussion and Troubleshooting

Since the digestion was successful, the Nuc DNA from well 2 and 3 could be purified from the gel and used for further experiments. In well 3, a double band can be seen between 500 bp and 800 bp. Only the band closer to 500 bp was purified from the gel. The concentration of Sample Nuc 2.1 was very low but further used for 3A Assembly.

2 3A assembly (ligation) of pSB1C3-T7-Nuc

Responsible

Ellinor Lindholm, Oscar He and Maren Maanja

Protocols used

3A Assembly (Sigma)

Modifications and comments to protocols

3A Assembly (Sigma): incubation for 14 h at 16 °C
Data of concentration of the T7 sample and volume of the DNA samples in the ligation mix are missing.

Experimental Setup

Table 5: Concentration and size of the parts used for the ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	14.8	2155
T7	-	55
Nuc 2.1	75.8	561
Nuc 2.2	73.8	561

Table 6: Volumes of the components used for ligation.

Component	Volume [μl]
10x T4 DNA Ligase Buffer (has ATP)	2
Vector DNA	-
Insert DNA (T7)	-
Insert DNA (Nuc)	-
T4 DNA Ligase	1
ddH_2O	to 20 ul

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{2}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ insert\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{3}$$

Results and Conclusions

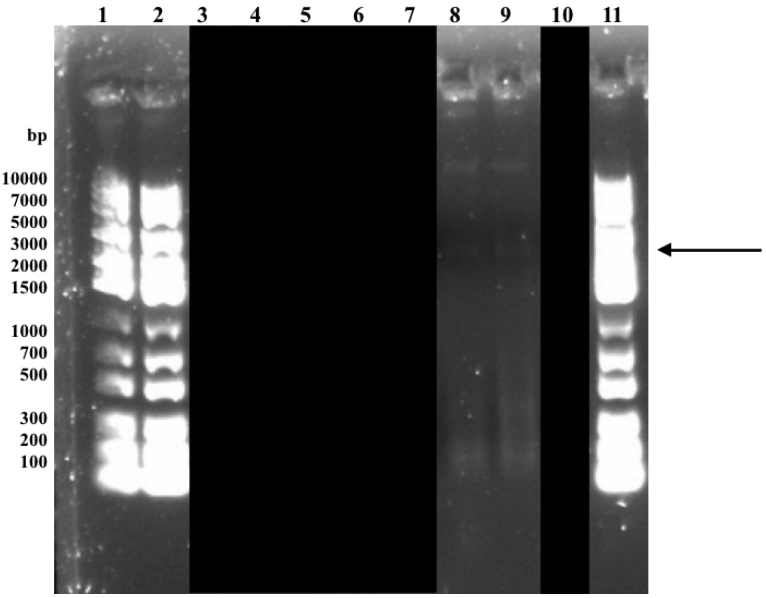

Figure 2: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) DNA Ladder (8) Nuc 2.1 (9) Nuc 2.2 (11) DNA Ladder.

Discussion and Troubleshooting

The gel showed a very faint band in well 8 and 9, corresponding to approximately the expected size of the pSB1C3-T7-Nuc. But the result is not enough to draw any conclusions. In order to test if the ligation was successful, the ligation product was transformed into Top10 and BL21(DE3) cells.

It was later (21/8-16) discovered that the pSB1C3 backbone used for this ligation was digested with XbaI and PstI instead of the correct EcoRI and PstI. This is a certain source of error which could explain the unsuccessful 3A assembly.

3 Transformation of pSB1C3-T7-Nuc in Top10 and BL21(DE3) cells with Colony PCR analysis and inoculation in liquid media

Responsible

Ellinor Lindholm, Oscar He and

Protocols used

Transformation
PCR Amplification

Modifications and comments to protocols

Q5 PCR Amplification primers:	VF2_R (reverse) VF2_F (forward)

Results and Conclusions

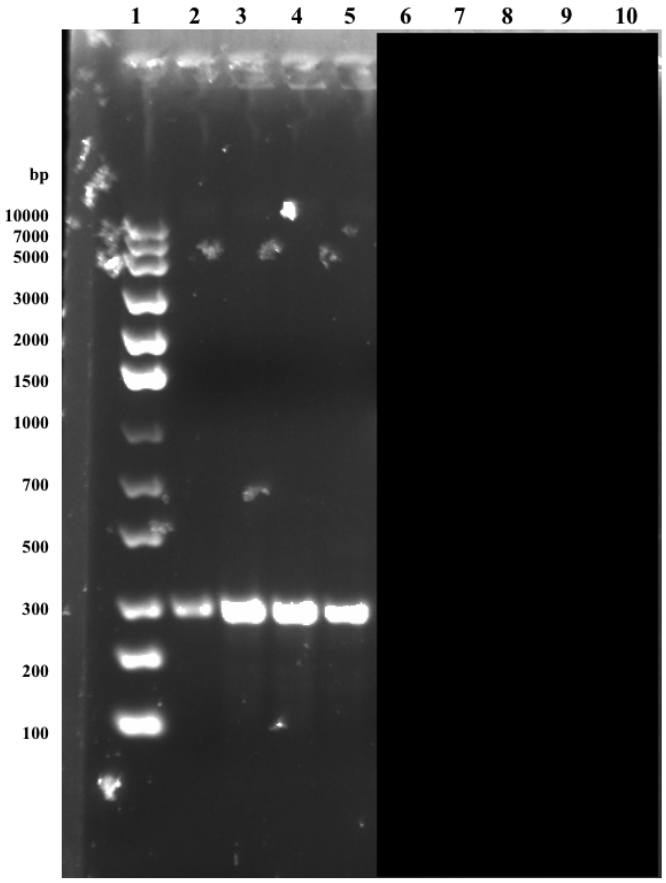

Figure 3: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 2.1 Top10 (3) Nuc 2.1 BL21(DE3) (4) Nuc 2.2 Top10 (5) Nuc 2.2 BL21(DE3).

The transformation was successful, however the sizes of the bands (around 300 bp) do not correspond to the expected size of 800-900 bp. The 3A assembly was unsuccessful.

Discussion and Troubleshooting

Because of the unsuccessful 3A assembly of pSB1C3-T7-Nuc, the samples were discarded and a 3A assembly were performed again with previously extracted nuclease plasmid samples.

4 PCR Amplification of pSB1C3-Nuc

Responsible

Ellinor Lindholm and Oscar He

Protocols used

PCR Amplification

Modifications and comments to protocols

Q5 PCR Amplification primers: VF2_R (reverse)
VF2_F (forward)

Experimental Setup

Table 7: Concentrations

Sample	Concentration [ng/μl]
Nuc 2.1 GS	56.3
Nuc 1 HQ	44.8

Results and Conclusions

Table 8: Concentrations of gel purified samples.

Sample	Concentration [ng/μl]
Nuc 2.1 GS	383.3
Nuc 1 HQ	408.25

Discussion and Troubleshooting

The PCR Amplification was successful and the Nuc samples Nuc 2.1 GS and Nuc 2.1 HQ was further used for digestion.

5 Digestion of pSB1C3-Nuc PCR Amplification product with PCR purification

Responsible

Ellinor Lindholm and Oscar He

Protocols used

3A Assembly (Sigma)
PCR Purification

Modifications and comments to protocols

3A Assembly (Sigma): total volume 20 μl (digestion mix)
3A Assembly (Sigma): 1 μg of DNA in digestion mix

Experimental Setup

Table 9: Concentrations of gel purified samples for digestion.

Sample	Concentration [ng/μl]
Nuc 2.1 GS	383.3
Nuc 1 HQ	408.25

Table 10: Volumes used for the digestion of the two pSB1C3-Nuc samples.

Component volumes	Nuc 2.1 GS [μl]	Nuc 1 HQ [μl]
DNA sample	2.6	2.44
10x SuRE/Cut Buffer H	2	2
XbaI	1	1
PstI	1	1
ddH2O	13.4	13.56

Table 11: Volumes of samples taken for PCR purification.

Sample	Volume [μl]
Nuc 2.1.1 GS	20
Nuc 2.1.2 GS	20
Nuc 1.1 HQ	20
Nuc 1.2 HQ	20

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (4)$$

Results and Conclusions

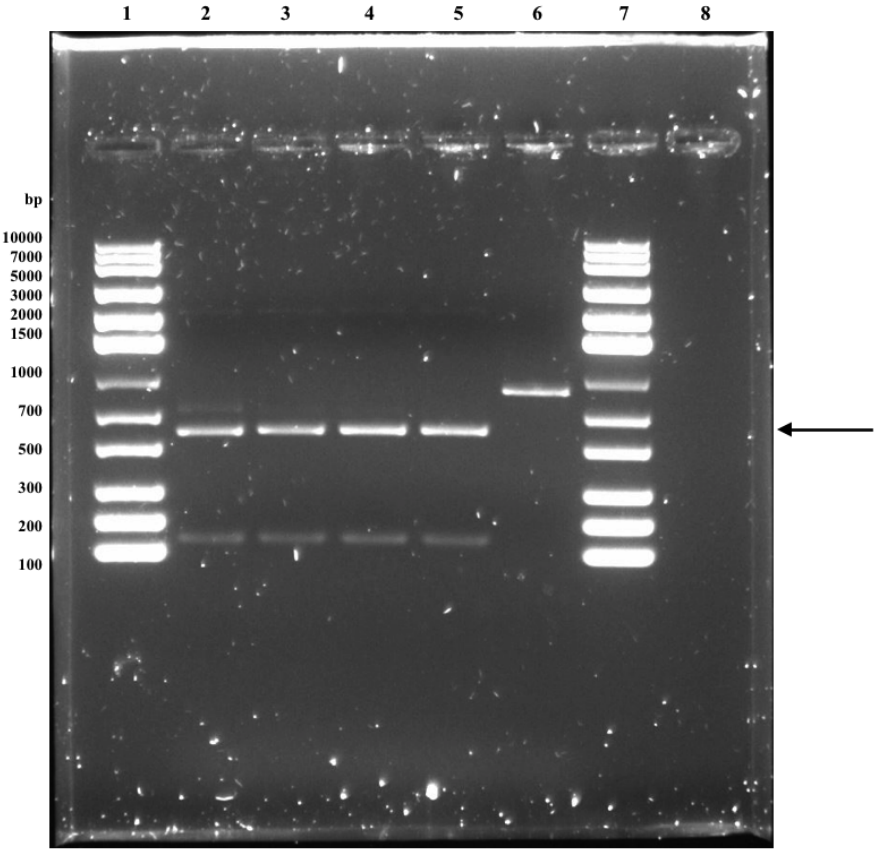

Figure 4: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 2.1.1 GS (3) Nuc 2.1.2 GS (4) Nuc 1.1 HQ (5) Nuc 1.2 HQ (6) Negative control (7) DNA Ladder.

The digestion was successful with the first band (top to bottom) corresponds to the correct size of approximately 560 bp.

Table 12: The concentration of the samples after PCR Purification, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc 2.1.1 GS	7.5
Nuc 2.1.2 GS	14.2
Nuc 1.1 HQ	7
Nuc 1.2 HQ	6.7

Discussion and Troubleshooting

Since the digestion was successful, the product could be used further in a 3A assembly with T7 and pSB1A3 after purification.

Since the band in 2 and 3 (around 300 bp) does not have the correct size, it was assumed that the ligation was unsuccessful.

6 3A assembly (ligation) of PCR purified product with PCR analysis

Responsible

Ellinor Lindholm and Oscar He

Protocols used

3A Assembly
Q5 PCR Amplification

Modifications and comments to protocols

	Amount of vector per reaction	29.6 ng
3A Assembly:	Ratio vector:insert	1:10
	Incubation	14 h at 16 °C
Q5 PCR Amplification:		1 μl DNA sample used in PCR mix.
Q5 PCR Amplification primers:		VF2_R (reverse)
		VF2_F (forward)

Experimental Setup

Table 13: Concentration and size of the parts used for the second attempt of ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	14.8	2155
T7	71.8	55
Nuclease (1.1 HQ)	7.0	561
Nuclease (2.1.2 GS)	14.2	561

Table 14: Volumes of each component used in the ligation mix.

Assemble part	1.1 HQ [μl]	2.1.2 GS [μl]
pSB1C3	2	2
T7	2	2
Nuclease (1.1 HQ)	21.1	10.4
T4 DNA Ligase	1	1
10x T4 DNA Ligase Buffer	2	2
ddH_2O	0	2.6

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1C3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{5}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{6}$$

Results and Conclusions

Table 15: Concentrations of the Nuc samples after 3A assembly and PCR purification.

Sample	Concentration [ng/μl]
Nuc 1.1 HQ	286.1
Nuc 2.1.2 GS	234.75

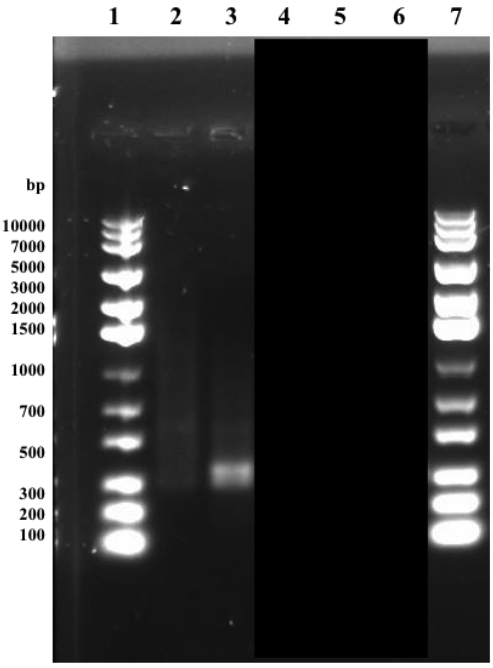

Figure 5: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 1.1 HQ (3) Nuc 2.1.2 GS (7) DNA Ladder.

The digested product does not have the correct size.

Discussion and Troubleshooting

The final amount of vector was set with regards to both the recommended range stated in the protocol and the vector sample concentration for an easy calculation.

It was later (21/8-16) discovered that the pSB1C3 backbone used for this ligation was digested with XbaI and PstI instead of the correct EcoRI and PstI. This is a certain source of error which could explain the unsuccessful 3A assembly.

7 Transformation of pSB1C3-T7-Nuc in Top10 and BL21(DE3) cells with Colony PCR analysis and inoculation in liquid media

Responsible

Ellinor Lindholm and Oscar He

Protocols used

Transformation
Q5 PCR Amplification

Modifications and comments to protocols

Q5 PCR Amplification primers: VF2_R (reverse)
VF2_F (forward)

Experimental Setup

Table 16: Volumes of liquid culture for each taken colony.

Sample	Volume [μl]
Nuc 2.1.2 GS Top10 1	3
Nuc 2.1.2 GS Top10 2	3
Nuc 2.1.2 GS Top10 3	3
Nuc 2.1.2 GS BL21 1	3
Nuc 2.1.2 GS BL21 2	3
Nuc 2.1.2 GS BL21 3	3
Nuc 1.1 HQ Top10	3
Nuc 1.1 HQ BL21 1	3
Nuc 1.1 HQ BL21 2	3
Nuc 1.1 HQ BL21 3	3

Results and Conclusions

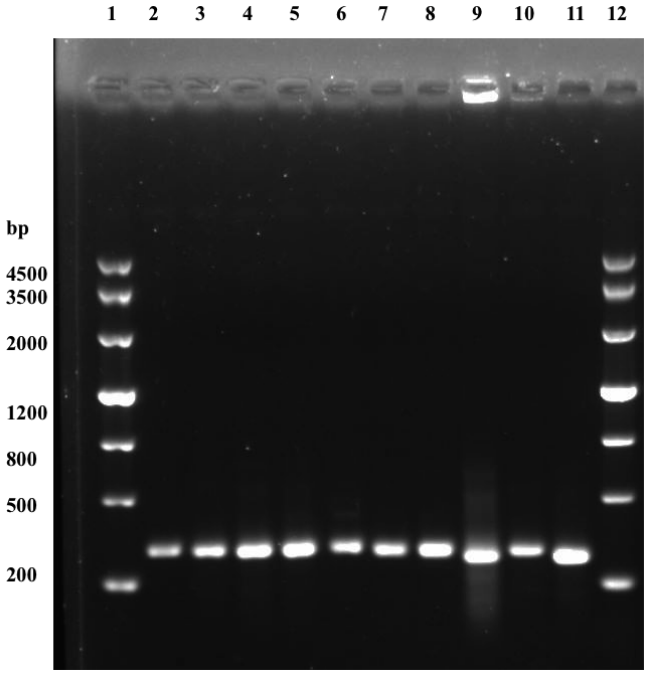

Figure 6: Result of the gel electrophoresis with 1 % agarose at 100 V.

Table 17: Well number for each sample.

Well #	1	2	3	4	5	6
Sample	DNA Ladder	Nuc 2.1.2 GS Top10 1	Nuc 2.1.2 GS Top 10 2	Nuc 2.1.2 GS Top10 3	Nuc 2.1.2 GS BL21(DE3) 1	Nuc 2.1.2 GS BL21(DE3) 2
Well #	7	8	9	10	11	12
Sample	Nuc 2.1.2 GS BL21(DE3) 3	Nuc 1.1 HQ Top10	Nuc 1.1 HQ BL21(DE3) 1	Nuc 1.1 HQ BL21(DE3) 2	Nuc 1.1 HQ BL21(DE3) 3	DNA Ladder

None of the samples have an expected size of 800-900 bp. The cells were transformed with the incorrect plasmid.

Discussion and Troubleshooting

Because of the unsuccessful ligation, the 3A assembly will be performed again.

8 3A assembly (ligation) of pSB1C3-T7-Nuc with PCR analysis

Responsible

Ellinor Lindholm and Oscar He

Protocols used

3A Assembly
PCR Amplification (Sigma)

Modifications and comments to protocols

	Amount of vector per reaction	23.0 ng
	Ratio vector:T7	1:10+
3A Assembly:	Ratio vector:Nuc(1.2 HQ)	1:2.9
	Ratio vector:Nuc(2.1.2 GS)	1:6.2
	Incubation:	14 h at 16 °C
PCR Amplification (Sigma):		Total volume 12.5 μl
		0.5 μl DNA sample used in PCR mix.
PCR Amplification (Sigma) primers:		VF2_R (reverse)
		VF2_F (forward)

Experimental Setup

Table 18: Concentration and size of the parts used for the second attempt of ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	11.5	2155
T7	71.8	55
Nuclease (1.2 HQ)	6.7	561
Nuclease (2.1.2 GS)	14.2	561

Table 19: Volumes of each component used in the ligation mix.

Assemble part	1.2 HQ [μl]	2.1.2 GS [μl]
pSB1C3	2	2
T7	10	10
DNA sample	10	10
T4 DNA Ligase	1	1
10x T4 DNA Ligase Buffer	2	2
ddH_2O	0	0

Table 20: Volumes of the components used in the PCR mix.

Component	Volume [μl]
PCR Grade Nucleotide Mix	0.25
10x Sigma PCR Buffer ($MgCl_2$)	1.25
VF2_F	0.625
VF2_R	0.625
Taq DNA Polymerase	0.25
DNA sample	0.5
ddH_20	9

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{7}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ insert\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{8}$$

Results and Conclusions

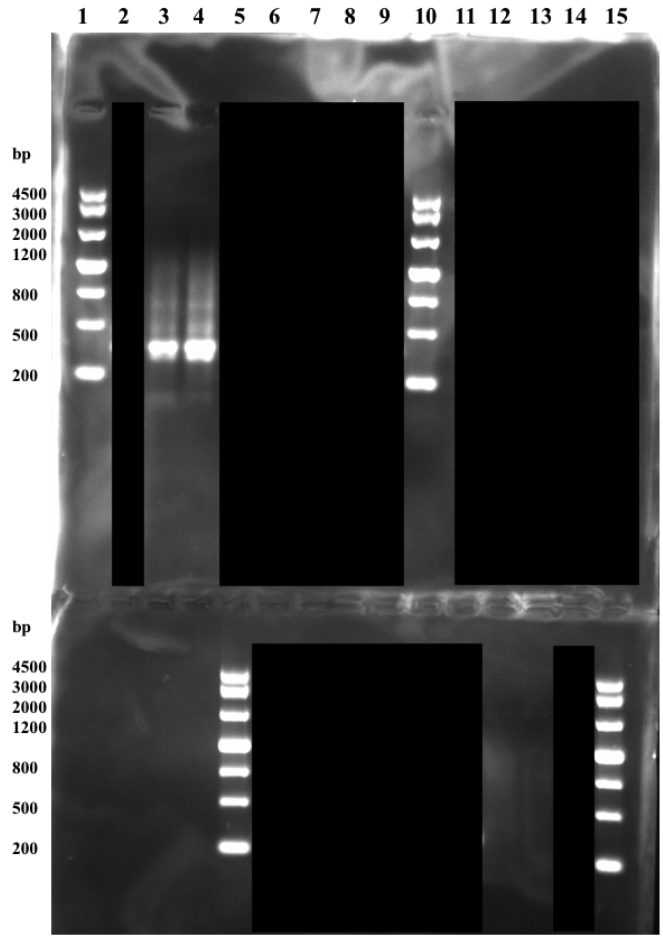

Figure 7: Result of the gel electrophoresis with 1 % agarose at 100 V. Top: (1) DNA Ladder (3) Nuc 2.1.2 GS lig prod PCR (4) Nuc 1.2 HQ lig prod PCR (10) DNA Ladder. Bottom: (5) DNA Ladder (12) Nuc 1.2 HQ lig prod (13) Nuc 2.1.2 GS lig prod (15) DNA Ladder.

No bands at expected sizes of 800-900 bp (PCR product) or 2800 bp (ligation product).

Discussion and Troubleshooting

The ligation was performed with a more than 1:10 T7:vector ratio. The amount of vector in the reaction was set both with regards to the recommended range stated in the protocol and the vector sample concentration to yield an easy calculation of the volume. It was intended to also have a 1:10 vector:nuclease ratio but since the sample concentrations were very low with a limited volume, only a maximum of 1:2.9 and 1:6.2 vector:nuclease ratio was possible for the samples 1.2 HQ and 2.1.2 GS respectively.

The gel electrophoresis on the PCR product of the ligation product and the ligation product itself shows that the desired plasmid is not present in the samples, leading to the conclusion of an unsuccessful 3A assembly (ligation).